THE INHIBITORY ACTION OF CERTAIN ORGANIC MERCURY COMPOUNDS ON THE GROWTH OF HUMAN TUBERCLE BACILLI

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. XXII

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Of all the chemicals so far tested by me during my study of the chemotherapy of tuberculosis, certain organic compounds of mercury have been found the most promising. About a year ago, a report [1] was made of the results so far obtained with about 20 mercury compounds used in experimental tuberculosis and the present report is based on work with 10 organic mercury compounds of phenol, nitro and nitroso phenols and saligenin or phenol carbinol; also on work with about 14 organic mercury compounds of aniline, the nitranilins, and methyl and ethyl anilins and nitranilins. A few other mercury compounds of the benzene nucleus have been used and the results are included in this report. Most of these preparations have been made for me in the Chemical Laboratory of the University of Chicago by Maurice Kharasch, Isadore Jacobsohn and Lyman Chalkley, to all of whom I am indebted for faithfulness and interest.

While my experiments with the organic mercurials were proceeding, Schamberg, Kolmer and Raiziss [2] published a report of a mercurial preparation made by them and called mercurophen or oxy-mercury-ortho-nitro phenolate. This is an interesting compound since it is freely soluble, contains about 53% of mercury, yet is several times as germicidal as mercuric chloride which contains 74% of mercury. Its phenol coefficient for different organisms is from 700 to 3,600 and even 10,000 in some reports. It is nonirritant to the skin, does not tarnish metallic instruments and is much less toxic than mercuric chloride. It was represented as especially valuable for the sterilization of the hands, skin, instruments, tubes, etc.[3] Since some of this compound was sent to me through the kindness of Dr. Schamberg, and since it is of the same type as some of my mercury nitro-phenol compounds, it was tested in the same way and is included in table 1 of this report.

In 1919, Young, White and Schwartz [4] developed an organic mercury compound of the dye fluorescein and called it mercurochrome-220. It contains

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[1] Jour. Infect. Dis., 1921, 28, p. 150.

[2] Ibid., 1919, 24, p. 547.

[3] Jour. Am. Med. Assn., 1917, 68, p. 1458.

[4] Ibid., 1919, 73, p. 1483. Lancaster, Burnett and Gaus: Ibid., 1920, 75, p. 721. Jour. Urol., 1921, 5, p. 353.

about 26% of mercury, is readily soluble and quite nonirritant and nontoxic. It kills B. coli in 15 minutes in a dilution of 1:5,000 and staphylococcus in a dilution of 1:10,000. It has been used with good effect in washing[4] out infected bladders, in open wounds, sinuses, and in treatment of infections of the throat, nose, ear and eye. Although unlike the other preparations here reported, it is, with mercurochrome 205 and 253, samples of which were also sent me by the kindness of Dr. White, included in table 3 of this report. A preliminary report on mercurophen and mercurochrome 220 in their action on tubercle bacilli and on experimental tuberculosis was made by me about a year ago.[5]

Hirschfelder[6] has made several organic mercury compounds of saligenin or phenol carbinol and has kindly furnished me with quantities sufficient for testing in my work. The results of my tests with these compounds also will be found in table 1.

In all my chemotherapeutic work it has been my custom to test first the power of the chemical being studied to inhibit the growth of the human tubercle bacillus in the test tube, not because we believe that therapeutic value is necessarily closely associated with inhibitory efficiency, but because it is the simplest method of getting a "lead" on the possible value of the compound and the advisability of testing it further. The chemicals which show a high inhibitory power are afterward tested on animals for their bactericidal and therapeutic efficiency. This paper reports only the inhibitory experiments. In every case, human tubercle bacilli of different degrees of virulence have been used, so that many of the tests have been repeated ten or twelve times. The tables give the extremes of variation when the different tests gave different results.

My method has been to add to tubes containing 10 c c of a sterile glycerol agar enough of the compound or of a stock solution of the compound to make dilutions from 1:100 up to 1:1,000,000. The tubes were then well shaken, slanted, cooled, and inoculated; they were examined after 15 days in the incubator, again after 30 days, and sometimes again after 45 days. The highest dilutions on which there was no growth at the end of the longest period allowed is recorded as the limit of "complete inhibition."

The mercury compounds of phenol in this table are all soluble in an alkaline solution so dilute that it does not itself prevent the growth of tubercle bacilli. Phenol itself has been found to inhibit growth of the human tubercle bacillus in a dilution of 1:1,000 but not at 1:5,000. Phenol with HgCl in the ortho position is 100 times as efficient in the

[5] Jour. Am. Med. Assn., 1920, 75, p. 1422.
[6] Jour. Am. Chem. Soc., 1920, 42, p. 2678.

TABLE 1
PHENOLS

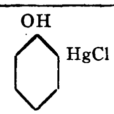
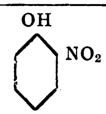
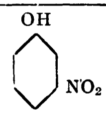
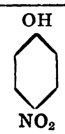
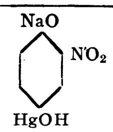
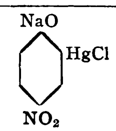
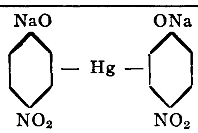
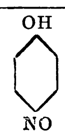
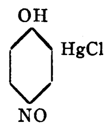
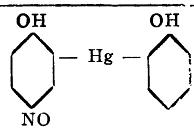
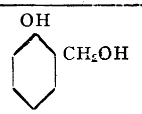
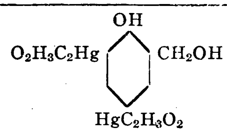
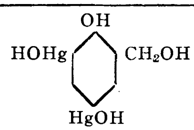
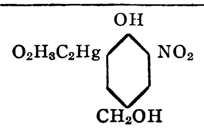
Name	Formula	Percentage of Mercury	Complete Inhibition
Phenol		0	1: 1,000
Phenol o-mercuric chloride		64.07	1: 100,000
Ortho-nitro phenol			1: 40,000 to 1: 50,000
Meta-nitro phenol			1: 5,000
Para-nitro phenol			1: 10,000 to 1: 20,000
Mercurophen Sodium o-nitro Phenol p-mercuric hydroxide		52.8	1: 50,000 to 1: 80,000
No. 9 Sodium p-nitro phenol o-mercuric chloride		52.8	1: 50,000 to 1: 100,000
No. 15 2,2′ mercuric bis p-nitro phenol		42	1: 100,000
P-nitroso phenol			1: 1,000 to 1: 5,000

TABLE 1—*Continued*

PHENOLS

Name	Formula	Percentage of Mercury	Complete Inhibition
P–nitroso phenol O–mercuric chloride		55.9	1: 80,000 to 1: 100,000
No. 20 2,2′ hydroxy 4–nitroso–mercury diphenyl		48.2	1: 100,000 to 1: 300,000
Saligenin phenol o–carbinol			1: 1,000
Phenol o–carbinol 2, 4 mercuric acetate		45.5	1: 20,000
Phenol o–carbinol 2, 4 mercuric hydroxide		74.4	1: 50,000 to 1: 60,000
O–nitro phenol P–carbinol O–mercuric acetate		46.9	1: 100,000

prevention of growth of the human tubercle bacillus as phenol and two and one half times as efficient as mercuric chloride, which inhibits in a dilution of 1:40,000. We find that the nitro-phenols are more efficient than phenol, the ortho-nitro-phenol being from 40 to 50 times as strong, the para-nitro-phenol from 10 to 20 times as strong and meta-nitro-phenol only 5 times as strong in its inhibitory action on the tubercle bacillus. The quinoidal change which is supposed to take place in the phenol molecule on the substitution of NO_2 for hydrogen probably accounts for this, since that change occurs most readily with NO_2 in the ortho position and least readily with it in the para position

Mercurophen and Na9 are isomers and the ortho position of the mercury in the latter seems more favorable than the para position. Na15 in which mercury forms a bridge between two sodium nitro phenols is stronger in its action than either of the other compounds, although its content of mercury is considerably less. In this, also, the bridge occupies the ortho position. Although p-nitroso phenol has lower inhibitory power than p-nitro-phenol, p-nitroso-phenol o-mercuric chloride has about the same power as Na9 which has the same chemical formula except that it has NO_2 in place of NO. Number 20, in which a phenol ring is substituted for the Cl and is connected to the p-nitroso-phenol by a mercury bridge in the ortho position, has greater inhibitory power than any of the other phenol compounds so far studied. The mercury saligenin compounds inhibited at from 1: 20,000 to 1: 60,000, although containing 45 to 74% of mercury, while the saligenin mercury compound which had one nitro group in the ortho position completely inhibits at 1: 100,000.

Table 2 contains the results of my inhibitory tests with a number of organic mercury compounds of aniline, nitro-anilines and methyl or ethyl anilines and nitranilines. Most of these compounds are insoluble except in dilute alcohol, which itself has considerable inhibitory power. Several of the mercury aniline and mercury methyl and ethyl anilines were combined with tartaric acid or acetic acid to form salts which are readily soluble in an alkaline solution so dilute that it does not itself check the growth of the organisms. The nitranilines, however, would not form soluble salts with any acids tested. The results as shown in table 2, seem to indicate that the addition of one or two methyl or of one or two ethyl groups does not materially affect the efficiency of these compounds as antiseptics and that the mercury is mostly, if not wholly, responsible for the fact that the mercury compounds of aniline are from 20 to 80 times as strong in their inhibitory action on the tubercle bacillus as aniline itself. On account of their insolubility, it has been necessary to use the nitro-compounds of aniline and mercury in alcoholic solutions, and even in alcohol they have not always been completely soluble down to dilutions of 1: 1,000. At 1: 10,000, however, they were soluble and in this dilution, the alcohol was sufficiently dilute to have very little if any antiseptic action, and growth has been completely prevented at 1: 100,000. The nitro group in the aniline molecule has not increased the inhibitory action of this substance as much as it did that of the phenol molecule, but the increase amounts to 5 to 10 times that of aniline. In the nitranilines, the meta

TABLE 2

ANILINES

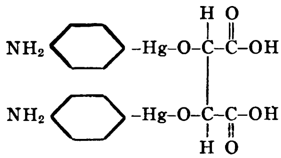
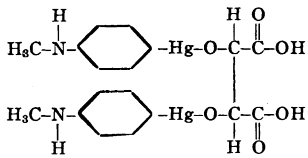
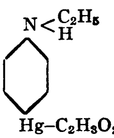
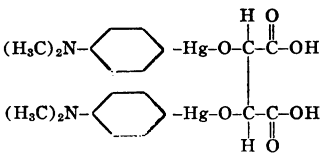
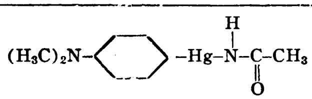
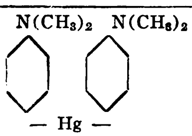
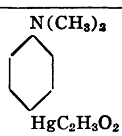
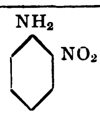
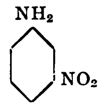
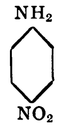
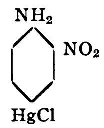
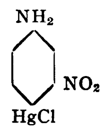
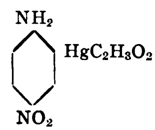
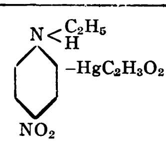
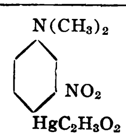
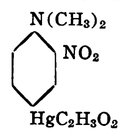
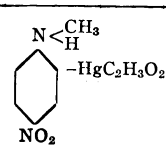
Name	Formula	Percentage of Mercury	Complete Inhibition
Aniline		0	1: 1,000
Aniline p–mercuric tartrate		53.9	1: 20,000 to 1: 40,000
Mono methyl aniline p—mercuric tartrate		50.7	1: 40,000 to 1: 80,000
Mono ethyl aniline p–mercuric acetate		52.8	1: 40,000 to 1: 60,000
40 A di methyl aniline p–mercuric tartrate		49.1	1: 20,000 to 1: 40,000
No. 37 Di methyl aniline p–mercuric acetamide		53.0	1: 40,000 to 1: 60,000
4, 4′ mercury bis di methyl aniline		45.5	1: 20,000
Di methyl aniline p–mercuric acetate		52.8	1: 20,000 to 1: 40,000
O–nitraniline		0	1: 5,000

TABLE 2—*Continued*

ANILINES

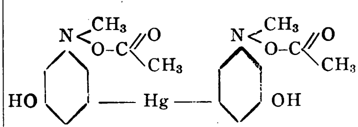
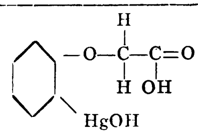
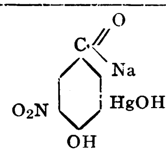
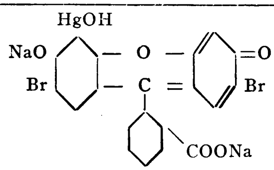
Name	Formula	Percentage of Mercury	Complete Inhibition
M–nitraniline		0	1: 10,000
P–nitraniline		0	1: 1,000 to 1: 5,000
O–nitraniline P–mercuric chloride		57.8	1: 100,000
M–nitraniline P–mercuric chloride		57.8	1: 100,000
P–nitraniline o–mercuric acetate		50.6	1: 50,000 to 1: 60,000
(14 M) Mono ethyl p–nitro aniline o–mercuric acetate		47.2	1: 100,000
No. 26 Di methyl m–nitro aniline p–mercuric acetate		47.2	1: 100,000
No. 27 Di methyl o–nitro aniline p–mercuric acetate		47.2	1: 100,000
No. 29 Mono methyl p–nitraniline o–mercuric acetate		48.8	1: 80,000 to 1: 100,000

position of the nitro group is most favorable instead of the ortho position as in the nitro-phenols. This is probably due to the fact that the quinoidal change in the ring does not easily take place in the aniline molecule.

Table 3 presents my results with several unrelated organic mercury compounds of the benzene nucleus. L. C. 27 is a very soluble mercury

TABLE 3

Other Mercury Compounds of the Benzene Nucleus

Name	Formula	Percentage of Mercury	Complete Inhibition
L. C. 27 Mercuric-bis 3-hydroxy phenyl trimethyl ammonium acetate		32	1: 1,000 to 1: 5,000
No. 55 Phenoxyl acetic acid o-mercury hydroxide		55.2	1: 10,000
No. 62 3 nitro - 4 hydroxyl 5-mercury-hydroxide benzoic acid		48.2	1: 20,000 to 1: 80,000
Mercurochrome-220		26.7	1: 10,000
Mercurochrome-205			1: 10,000
Mercurochrome-253			1: 10,000

bridge compound containing only 32% of mercury. It shows, however, very little more inhibitory action than phenol. Number 55 is soluble, contains 55% of mercury and inhibits at 10,000, while No. 62, a similar compound but having a nitro group ortho to the hydroxyl and with only 48% of mercury, inhibits at 1:80,000. Three modifications of the mercurialized fluorescein compound made by Young, White and Schwartz all have the same inhibitory power, 1:10,000.

SUMMARY

The power of phenol to inhibit the growth of the human tubercle bacillus is greatly increased by the substitution of a mercury salt in place of one of the hydrogens,—hence a mercury united by one bond to carbon.

It is also increased by the substitution of one NO_2 group for one hydrogen in the ring.

The position of the NO_2 group has much to do with the degree of increase of the inhibitory power, the ortho position being most favorable and the para position next. This is probably due to a quinoidal change in the phenol nucleus.

The position of the mercury group also has much influence on the degree of increase of inhibitory power, the ortho position seeming most favorable.

The mercury bridge compounds seem also to have a high inhibitory power, at least in the two compounds tested, in both of which the bridge occupies the ortho position.

Although saligenin or phenol carbinol has the same inhibitory power as phenol, the mercury derivatives of this have a greatly increased efficiency, varying somewhat with the percentage of mercury; one compound, however· which has less mercury but in which both a nitro and a mercury group occupy the ortho position with respect to the hydroxy group, has a higher inhibitory power.

In the aniline compounds also the substitution of a mercury group greatly increases the efficiency.

The nitro group also increases the inhibitory efficiency but not in the same order of position as in the nitro-phenols, since the quinoid change does not readily take place in the aniline nucleus. However, the aniline compounds having the nitro group in the ortho position and the mercury salt in the para position seem more efficient than if the order is reversed.

Methyl and ethyl groups do not materially affect the antiseptic power of the aniline compounds, although these compounds having methyl or ethyl groups plus nitro groups plus mercury groups have a very high antiseptic power, not apparently varying much either with the percentage of mercury or with the relative position of the different groups.